PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 01 March 2001 (01.03.01)	
International application No.: PCT/KR99/00488	Applicant's or agent's file reference: PO-99171
International filing date: 26 August 1999 (26.08.99)	Priority date:
Applicant: LEE, Hyo, Hoon et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

26 June 2000 (26.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PO-99171

Box No. I TITLE OF INVENTION

Microorganisms and methods for producing threonine

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Daesang Corporation

52-1, Kayang-dong, Kangseo-ku, Seoul 157-200
Republic of Korea

☐ This person is also inventor.

Telephone No. 02-220-9500

Facsimile No. 02-232-3719

Teleprinter No.

State *(that is, country)* of nationality: KR

State *(that is, country)* of residence: KR

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LEE, Hyo Hoon

Jukong Apt. 117-1401,
Anheung-dong, Icheon, Kyongki-do 467-050
Republic of Korea

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is,country)* of nationality: KR

State *(that is, country)* of residence: KR

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☐ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HWANG, E-Nam

3rd Follr, Yegun Building, 823-42, Yoksam-dong,
Kangnam-ku, Seoul 135-080
Republic of Korea

Telephone No. 02-567-6562

Facsimile No 02-557-3101

Teleprinter No.

☐ **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HAN, Jae Chun

Misung Apt. 1-209, 17, Wolge 3-dong,
Nowon-ku, Seoul 139-771
Republic of Korea

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: KR

State *(that is, country)* of residence: KR

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

JUNG, Tae Man

Kunyoung Apt. 104-1201, 515-2, Joongkye 3-dong,
Nowon-ku, Seoul 139-223
Republic of Korea

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: KR

State *(that is, country)* of residence: KR

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*.

Regional Patent

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*

- ☒ AE United Arab Emirates
- ☒ AL Albania
- ☒ AM Armenia
- ☒ AT Austria
- ☒ AU Australia
- ☒ AZ Azerbaijan
- ☒ BA Bosnia and Herzegovina
- ☒ BB Barbados
- ☒ BG Bulgaria
- ☒ BR Brazil
- ☒ BY Belarus
- ☒ CA Canada
- ☒ CH and LI Switzerland and Liechtenstein
- ☒ CN China
- ☒ CU Cuba
- ☒ CZ Czech Republic
- ☒ DE Germany
- ☒ DK Denmark
- ☒ EE Estonia
- ☒ ES Spain
- ☒ FI Finland
- ☒ GB United Kingdom
- ☒ GD Grenada
- ☒ GE Georgia
- ☒ GH Ghana
- ☒ GM Gambia
- ☒ HR Croatia
- ☒ HU Hungary
- ☒ ID Indonesia
- ☒ IL Israel
- ☒ IN India
- ☒ IS Iceland
- ☒ JP Japan
- ☒ KE Kenya
- ☒ KG Kyrgyzstan
- ☒ KP Democratic People's Republic of Korea
- ☒ KR Republic of Korea
- ☒ KZ Kazakhstan
- ☒ LC Saint Lucia
- ☒ LK Sri Lanka
- ☒ LR Liberia
- ☒ LS Lesotho
- ☒ LT Lithuania
- ☒ LU Luxembourg
- ☒ LV Latvia
- ☒ MD Republic of Moldova
- ☒ MG Madagascar
- ☒ MK The former Yugoslav Republic of Macedonia
- ☒ MN Mongolia
- ☒ MW Malawi
- ☒ MX Mexico
- ☒ NO Norway
- ☒ NZ New Zealand
- ☒ PL Poland
- ☒ PT Portugal
- ☒ RO Romania
- ☒ RU Russian Federation
- ☒ SD Sudan
- ☒ SE Sweden
- ☒ SG Singapore
- ☒ SI Slovenia
- ☒ SK Slovakia
- ☒ SL Sierra Leone
- ☒ TJ Tajikistan
- ☒ TM Turkmenistan
- ☒ TR Turkey
- ☒ TT Trinidad and Tobago
- ☒ UA Ukraine
- ☒ UG Uganda
- ☒ US United States of America
- ☒ UZ Uzbekistan
- ☒ VN Viet Nam
- ☒ YU Yugoslavia
- ☒ ZA South Africa
- ☒ ZW Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☐
- ☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn bythe applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA / AT

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	04
description (excluding sequence listing part)	:	13
claims	:	02
abstract	:	01
drawings	:	
sequence listing part of description	:	
Total number of sheets	:	20

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☒ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request)

HWANG, E-Nam

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
HWANG, E-Nam
PARK, Hyong Joon
3rd Floor, Yegun Building,
823-42, Yoksam-dong, Kangnam-ku
Seoul 135-080
Republic of Korea

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing (day/month/year)	30 November 2001 (30.11.01)
Applicant's or agent's file reference: PO-99171	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority Date (day/month/year)
PCT/ KR 99/00488	26 August 1999 (26.08.99)	

Applicant
DAESANG CORPORATION et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the eleceted Offices.

3. Where required by any of the elected Offices, the Interational Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the eleceted Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/AT	Authorized officer
Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/200	Wolf Telephone No. +43 / 1 / 53424 - 450

Form PCT/IPEA/416 (July 1992)

ASIANA INTERNATIONAL PATENT&LAW OFFICE 2002. 1. 04

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PO-99171		
International application No.	International filing date (*day/month/year*)	Priority Date (*day/month/year*)
PCT/KR 99/00488	26 August 1999 (26.08.1999)	
International Patent Classification (IPC) or national classification and IPC		
IPC[7]: C12N 1/20; C12P 13/08 // (C12N 1/20; C12R 1:19)		
Applicant		
DAESANG CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examination Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   - I. ☒ Basis of the opinion
   - II. ☐ Priority
   - III. ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV. ☐ Lack of unity of invention
   - V. ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI. ☐ Certain documents cited
   - VII. ☐ Certain defects in the international application
   - VIII. ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 June 2000 (26.06.2000)	30 October 2001 (30.10.2001)
Name and mailing address of the IPEA/AT Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer MOSSER Telephone No. 1/53424/437

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00488

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☐ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____.

☐ the claims, Nos. _____.

☐ the drawings, sheets/fig _____.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00488

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-8	YES
	Claims		NO
Inventive step (IS)	Claims	1-8	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-8	YES
	Claims		NO

Citations and explanations (Rule 70.7)

The documents cited in the search report are merely interesting contributions to the state of the art and do not interfere with novelty and inventive step of the subject-matters of present claims 1-8. The industrial applicability is obvious for the subject-matters of all claims.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PO-99171		
International application No.	International filing date (*day month year*)	Priority Date (*day month year*)
PCT/KR 99/00488	26 August 1999 (26.08.1999)	
International Patent Classification (IPC) or national classification and IPC		
IPC[7]: C12N 1/20; C12P 13/08 // (C12N 1/20; C12R 1:19)		
Applicant		
DAESANG CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examination Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I. ☒ Basis of the opinion
- II. ☐ Priority
- III. ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV. ☐ Lack of unity of invention
- V. ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI. ☐ Certain documents cited
- VII. ☐ Certain defects in the international application
- VIII. ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 June 2000 (26.06.2000)	30 October 2001 (30.10.2001)
Name and mailing address of the IPEA/AT Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer MOSSER Telephone No. 1/53424/437

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00488

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☐ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____.

☐ the claims, Nos. _____.

☐ the drawings, sheets/fig _____.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00488

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-8	YES
	Claims		NO
Inventive step (IS)	Claims	1-8	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-8	YES
	Claims		NO

Citations and explanations (Rule 70.7)

The documents cited in the search report are merely interesting contributions to the state of the art and do not interfere with novelty and inventive step of the subject-matters of present claims 1-8. The industrial applicability is obvious for the subject-matters of all claims.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14525 A1

(51) International Patent Classification[7]: C12N 1/20, C12P 13/08 // (C12N 1/20, C12R 1:19)

(21) International Application Number: PCT/KR99/00488

(22) International Filing Date: 26 August 1999 (26.08.1999)

(25) Filing Language: English

(26) Publication Language: English

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(54) Title: MICROORGANISMS AND METHODS FOR PRODUCING THREONINE

(57) Abstract: The present invention relates to methods and microorganisms for producing L-threonine. In particular, the present invention relates to the production of L-threonine using microorganisms, and *Escherichia coli* strains in particular, which require L-methionine for growth and are L-isoleucine-leaky, are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, medium containing 60 % of L-threonine fermentation mother liquid, azetidine and dehydroproline, and which are susceptible to fluoropyruvate.

MICROORGANISMS AND METHODS FOR PRODUCING THREONINE

Background of the Invention

Field of the Invention

The present invention relates to microorganisms and methods for producing L-threonine. In particular, the present invention relates to the production of L-threonine using microorganisms, and *Escherichia coli* strains in particular, which require L-methionine, are L-isoleucine-leaky for growth, are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, medium containing 60% of L-threonine fermentation mother liquid (which contains more than 9.0% threonine), azetidine and dehydroproline, and which are susceptible to fluoropyruvate.

Description of the Related Art

L-threonine, an essential amino acid, is a second limited amino acid of rice. As is well known, L-threonine is used as a component for, e.g., amino acid transfusion liquid or general amino acid tablets, and as a nutrient. Recently, there has been a great increase in the demand for L-threonine because it, together with L-lysine, is used as an additive in feedstuff.

Japanese Pat. Publication No. Heisei 5-10076 teaches use of recombinant DNA which contains the genetic information for asparto kinase, homoserine kinase, homoserine dehydrogenase, and threonine synthase in production of threonine from a L-threonine-producing *Serratia* sp. Japanese Pat. Publication No. Heisei 1-289493 discloses that a DNA taken from a *Providencia* sp. resistant to methionine metabolic antagonist is genetically engineered and used to increase the productivity of L-threonine. In order to produce L-threonine, a threonine metabolic antagonist-resistant *Escherichia* sp. which requires methionine or diaminopimelic acid for growth has been used (Japanese Pat. Publication No. Sho. 56-10037). A strain which can grow in medium containing L-serine and ethionine has also been used (EP 91103569.9).

Summary of the Invention

The present invention relates to novel microorganisms and methods of producing large quantities of L-threonine using the microorganisms. The present microorganisms are derived from the microorganism deposited in the Korean Culture Center of Microorganisms, College of Engineering, Yonsei University, Sodaemun gu, Seoul 120-749,

Republic of Korea, on July 16, 1998, having the deposition number KCCM-10132. KCCM-10132 has also been described in PCT Application Number PCT/KR 98/00340.

KCCM-10132, the parent strain of the present microorganisms, requires both L-methionine and L-isoleucine, is resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid and L-threonine, and is susceptible to fluoropyruvate. KCCM 10132 also requires diaminopimelic acid.

To obtain the present microorganisms, KCCM-10132 was mutated and cells which were resistant to L-threonine, able to grow on medium containing 60% of L-threonine fermentation mother liquid (which contains more than 9.0% threonine), azetidine and dehydroproline were selected. As used herein, the terminology "able to grow on medium containing L-threonine fermentation mother liquid" means that the microorganism is able to grow on minimal agar plates which contain the production medium for the parent strain KCCM10132, the ingredients of which are listed in Example 2. The production medium for the parent strain KCCM 10132 contains glucose at a concentration of 10%, corn steep liquor at a concentration of 3%, potassium dihydrogen phosphate at a concentration of 0.1%, ferrous sulfate at a concentration of 2 ㎎/ ℓ , manganese sulfate at a concentration of 2 ㎎/ ℓ , ammonium sulfate at a concentration of 0.05%, urea at a concentration of 0.6%, L-methionine at a concentration of 200 ㎎/ ℓ and pH 7.0 and L-isoleucine at a concentration of 200 ㎎/ ℓ . To prepare the "L-threonine fermenation mother liquid" the parent strain KCCM10132 is grown in the preceding medium for 100 hours at 30 degrees Celsius. When cultured in media containing a high concentration of glucose, the present microorganisms accumulate large quantities of L-threonine in the culture.

Detailed Description of the Preferred Embodiments

The novel strain of the present invention, which has been deposited in the Korean Culture Center of Microorganisms and has been assigned deposit number KCCM-10168, can grow well in the presence of fermentation mother liquid in which the strain was grown, L-azetidine-2-carboxylic acid and 3,4-dehydro-DL-proline. In other words, KCCM-10168 is resistant to fermentation mother liquid in which the strain was grown, L-azetidine-2-carboxylic acid (hereinafter "azetidine") and 3,4-dehydro-DL-proline(hereinafter "dehydroproline").

As discussed above, KCCM-10168 was derived from a strain deposited at the

Korean Culture Center of Microorganisms under deposit number KCCM-10132. The strains of the present invention may be obtained by treating KCCM-10132 with UV or with chemical mutagens, such as NTG(N-methyl-N'-nitro-N-nitroso guanidine) and DES(diethylsulfate). Following mutagenesis, the cells were streaked on minimal agar plates containing 60% of L-threonine fermentation mother liquid(more than 9.0% of threonine) to select L-threonine fermentation mother liquid-resistant colonies.

The selected colonies were streaked on complete agar plates containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline and cultured at 37 ℃ for 2~3days. The complete agar plates included yeast extract 1.0%, peptone 1.0%, beef broth 0.3%, NaCl 0.5% and glucose 0.5% at pH 7.0.

Thereafter, replicas of the colonies grown were made on a minimal agar plate containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline and a minimal agar plate devoid of these components. Of the colonies which survived on the agar plate containing 60% of L-threonine fermentation mother liquid, 2g/ℓ azetidine and 2g/ℓ dehydroproline, those which were clearly grown were separated, and their characteristics were compared with those of the parent strain KCCM-10132. The minimal plates on which the microbiological properties of the mutant and parent strains were compared included 1.0% glucose, 0.2% ammonium sulfate, 0.1% potassium dihydrogen phosphate, 0.02% magnesium sulfate at pH 7.3, and 2% agar . Diaminopimelic acid at 100 ㎎/ℓ, L-methionine at 200 ㎎/ℓ and L-isoleucine at 200 ㎎/ℓ were respectively used in order to determine whether they were needed for the growth of the novel strain.

DSM9906 is a strain obtained using the above procedure. DSM9906 was deposited in the Korea Culture Center of Microorganisms, College of Engineering, Yonsei University, Sodaemun gu, Seoul 120-749, Republic of Korea, on July 29, 1999 and was assigned Deposition No. KCCM-10168. While the following discussion utilizes KCCM-10168 as an example of the microorganisms of the present invention, it will be appreciated that other strains, and in particular other *E. coli* strains, which have the properties of KCCM-10168 may be used to produce L-threonine. In particular, the *E. coli* strains may be derived from KCCM-10132. Alternatively, the microorganisms may be from species other than *E. coli*, including strains of *Brevibacteria* or *Corynebacteria*.

For example, to select a microorganism which requires L-methionine for growth,

the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-methionine in order to identify colonies which grow in the presence of L-methionine but not in the absence of L-methionine. The L-methionine may be present at any concentration which is sufficient to differentiate strains which require L-methionine from strains which do not require L-methionine. For example, the L-methionine may be present at from about 50 mg/ℓ to about 400 mg/ℓ, preferably from about 100 mg/ℓ to about 300 mg/ℓ, and more preferably at about 200 mg/ℓ. Strains which grow in the presence of L-methionine but not in its absence may be used in conjunction with the present invention.

For example, to select a microorganism which is leaky for L-isoleucine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth and 2% agar at pH 7.3 with or without L-isoleucine in order to identify colonies which grow slowly in the absence of L-isoleucine but which exhibit more robust growth in the presence of L-isoleucine. The L-isoleucine may be present at any concentration which is sufficient to identify strains which grow slowly in the absence of L-isoleucine from strains but which exhibit more robust growth in its presence. For example, growth on plates containing L-isoleucine at from about 50 mg/ℓ to about 400 mg/ℓ, preferably from about 100 mg/ℓ to about 300 mg/ℓ, and more preferably at about 200 mg/ℓ can be compared to growth on plates lacking L-isoleucine. Strains which grow slowly in the absence of L-isoleucine but more robustly in its presence may be used in conjunction with the present invention.

To select a microorganism which is resistant to α-methylserine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete

agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, α-methylserine and 2% agar at pH7.0. The α-methylserine may be present at any concentration which is sufficient to differentiate strains which are resistant to α-methylserine from strains which are sensitive to α-methylserine. For example, growth on plates containing α-methylserine at from about 10mM to about 200mM, preferably from about 20mM to about 100mM, and more preferably at about 40mM indicates that the strain is resistant to α-methylserine. The resulting colonies are then replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without α-methylserine at the concentration used in the complete agar plates in order to identify colonies which are resistant to α-methylserine. Strains which grow in the presence of α-methylserine may be used in conjunction with the present invention.

To select a microorganism which is resistant to diaminosuccinic acid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, diaminosuccinic acid and 2% agar at pH7.0. The diaminosuccinic acid may be present at any concentration which is sufficient to differentiate strains which are resistant to diaminosuccinic acid from strains which are sensitive to diaminosuccinic acid. For example, growth on plates containing diaminosuccinic acid at from about 0.5g/L to about 50g/L, preferably from about 1 g/L to about 10g/L, and more preferably at about 2.5g/L indicates that the strain is resistant to diaminosuccinic acid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without diaminosuccinic acid at the concentration used in the complete agar plates in order to identify colonies which are resistant to diaminosuccinic acid. Strains which grow in the presence of diaminosuccinic acid may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-glutamic acid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5%

glucose, L-glutamic acid and 2% agar at pH7.0. The L-glutamic acid may be present at any concentration which is sufficient to differentiate strains which are resistant to L-glutamic acid from strains which are sensitive to L-glutamic acid. For example, growth on plates containing L-glutamic acid at from about 50mM to about 500mM, preferably from about 100mM to about 300mM, and more preferably at about 240mM indicates that the strain is resistant to L-glutamic acid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-glutamic acid at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-glutamic acid. Strains which grow in the presence of L-glutamic acid may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-threonine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, L-threonine and 2% agar at pH7.0. The L-threonine may be present at any concentration which is sufficient to differentiate strains which are resistant to L-threonine from strains which are sensitive to L-threonine. For example, growth on plates containing L-threonine at from about 1% to about 13%, preferably from about 3% to about 10%, and more preferably at about 7% indicates that the strain is resistant to L-threonine. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-threonine at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-threonine. Strains which grow in the presence of L-threonine may be used in conjunction with the present invention.

To select a microorganism which is resistant to L-threonine fermentation mother liquid, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, L-threonine fermentation mother liquid and 2% agar at pH7.0. The L-threonine fermentation mother liquid may be present at any concentration which is sufficient to differentiate strains which are resistant to L-threonine fermentation mother liquid from strains which are sensitive to L-threonine fermentation mother liquid.

For example, the plates may contain from about 20 to about 80%, more preferably from aobut 40 to about 70%, and in particular 60% L-threonine fermentation mother liquid. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without L-threonine fermentation mother liquid at the concentration used in the complete agar plates in order to identify colonies which are resistant to L-threonine fermentation mother liquid. Strains which grow in the presence of L-threonine fermentation mother liquid may be used in conjunction with the present invention.

To select a microorganism which is resistant to azetidine, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, azetidine and 2% agar at pH7.0. The azetidine may be present at any concentration which is sufficient to differentiate strains which are resistant to azetidine from strains which are sensitive to azetidine. For example, growth on plates containing azetidine at from about 0.5 g/ ℓ to about 5 g/ ℓ , preferably from about 1 g/ ℓ to about 3 g/ ℓ , and more preferably at about 2 g/ ℓ indicates that the strain is resistant to azetidine. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without azetidine at the concentration used in the complete agar plates in order to identify colonies which are resistant to azetidine. Strains which grow in the presence of azetidine may be used in conjunction with the present invention.

To select a microorganism which is resistant to dehydroproline, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on complete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, dehydroproline and 2% agar at pH7.0. The dehydroproline may be present at any concentration which is sufficient to differentiate strains which are resistant to dehydroproline from strains which are sensitive to dehydroproline. For example, growth on plates containing dehydroproline at from about 0.5 g/ ℓ to about 5 g/ ℓ , preferably

from about 1 g/ ℓ to about 3 g/ ℓ , and more preferably at about 2 g/ ℓ indicates that the strain is resistant to dehydroproline. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without dehydroproline at the concentration used in the complete agar plates in order to identify colonies which are resistant to azetidine. Strains which grow in the presence of dehydroproline may be used in conjunction with the present invention.

To select a microorganism which is sensitive to fluoropyruvate, the microorganism is mutagenized as described above. After mutagenesis, the cells are streaked on compiete agar plates comprising 1% yeast extract, 1% peptone, 0.3% beef broth, 0.5% NaCl, 0.5% glucose, and 2% agar at pH7.0. The colonies are replica plated on minimal plates containing 5.0% fructose, 1.4% ammonium sulfate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulfate, a sufficient amount of any additional compounds required by the microorganism to permit growth, and 2% agar at pH 7.3 with or without fluoropyruvate. The fluoropyruvate may be present at any concentration which is sufficient to differentiate strains which are sensitive to fluoropyruvate from strains which are resistant to fluoropyruvate. For example, lack of growth on plates containing fluoropyruvate at from about 10mM to about 200mM, preferably from about 20mM to about 100mM, and more preferably at about 40mM indicates that the strain is sensitive to fluoropyruvate. Strains which grow in absence of fluoropyruvate but not in the presence of fluoropyruvate may be used in conjunction with the present invention.

It will be appreciated that each of the above selection procedures need not be performed in individual steps. Instead, after mutagenesis, the microorganisms may be plated on complete agar which selects for more than one characteristic simultaneously. For example, two or more characteristics may be simultaneosly selected. For example, to select cells which are resistant to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, L-threonine fermentation mother liquid, azetidine, and dehydroproline, these each of these compositions may be included in the complete agar plates on which the mutagenized cells are streaked at the concentrations provided above. Thereafter, the mutagenized cells may be plated on minimal agar plates containing each of these compositions at the concentrations provided above.

It will also be appreciated that mutagenesis need not be separately performed for each characteristic to be selected. Rather, after mutagenesis, microorganisms which have a desired characteristic may be identified as provided above. Thereafter, the identified microorganisms may be selected for additional desired characteristics as described above.

As shown in Table 1, the novel strain KCCM-10168 is resistant to 60% of L-threonine fermentation mother liquid, 2g/ ℓ azetidine and 2g/ ℓ dehydroproline. As shown in Table 2, KCCM-10168 retains most of the characteristics of the parent strain, including a requirement for L-methione and resistance to L-threonine and L-glutamic acid. However, unlike the parent strain, KCCM-10168 is isoleucine-leaky and does not require diaminopimelic acid.

Table 1. Growth of the novel strain KCCM-10168 in the broths containing 60% of L-threonine fermentation mother liquid, azetidine and dehydroproline.

Concentration			Strains	
L-threonine fermentation mother liquid(%)	Azetidine(g/ ℓ)	Dehydroproline (g/ ℓ)	Parent strain (KCCM-10132)	novel strain DSM9906 (KCCM-10168)
0	0	0	1.804	1.821
60	1	1	0.053	1.675
	2	2	0.048	1.587
	4	4	0.049	0.501
80	1	1	0.058	0.092
	2	2	0.064	0.089
	4	4	0.059	0.077

Note: Growth of the strains was measured by absorbance at 610 ㎚ after culturing them for 24 hours in minimal broth containing above three compounds.

Table 2. The comparison of the characteristics of DSM9906(KCCM-10168) and Parent strain (KCCM-10132).

Concentration						Strains			
L-Met (mg/ℓ)	L-Ile (mg/ℓ)	DAPA (mg/ℓ)	Fer. Liquid 60%	Azeti.	De Hydro proline	Parent strain (KCCM-10132)		DSM9906 (KCCM-10168)	
						Min. broth	Com-plete broth	Min. broths	Com-plete broth
200	-	-	-	-	-	-	+++	+	+++
-	200	-	-	-	-	-	+++	-	+++
200	200	-	-	-	-	+	+++	+++	+++
200	200	100	-	-	-	+++	+++	+++	+++
200	200	100	add	2g/ℓ	2g/ℓ	-	+	+++	+++

Note : Growth state after being cultured in broth containing above six compounds.
(-:no growth, +:growth, ++:good growth, +++:very good growth)

The growth and yield of L-threonine obtained with DSM9906 (KCCM-10168) was compared to those obtained with the parent strain KCCM-10132 at different glucose concentrations and the results are shown in Table 3. As shown in Table 3, KCCM-10168 provided a greater yield of L-threonine than KCCM-10132 in a high concentration of glucose.

Table 3. Comparison of the growth and productivity between DSM9906 (KCCM-10168) and KCCM-10132.

L-Glu Conc.		Strains	
		KCCM-10132	DSM9906 (KCCM-10168)
5.0%	Growth[1]	0.555	0.567
	L-threonine[2]	12.53	12.61
7.0%	Growth	0.608	0.613
	L-threonine	16.92	17.34
10.0%	Growth	0.852	0.866
	L-threonine	19.86	22.97
12.5%	Growth	0.590	0.861
	L-threonine	13.09	22.23

Note[1] : 50-fold diluted solutions of the cultures incubated for 36~72 hours in production media(Example 1) were measured by absorbance at 610nm(Beckman DU-70)
Note[2] : L-threonine accumulated in cultures was measured using an automatic amino acid analyzer(Hitachi L-8500A)

Example 1 Production of L-threonine using DSM9906 (KCCM-10168)

- Strain used : DSM9906 (KCCM-10168)

• Pre-culture medium composition : Glucose 0.5%, Yeast Extract 1.0%, Peptone 1.0%, NaCl 0.5%, Beef broth 0.3%, pH 7.0

• Production medium composition : Glucose 12.5%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ℓ, Manganese sulfate 2 mg/ℓ, Ammonium sulfate 0.5%, L-Methionine 200 mg/ℓ and Calcium carbonate 5%(separately sterilized), pH 7.0. In the case of the parent strain KCCM-10132, L-Isoleucine was added at 200 mg/ℓ.

• Pre-Culturing : 5 mℓ of the pre-culture medium was aliquoted to 18Φ×185mm test tubes and autoclaved at 121 ℃ for 15min. under pressure. After being cooled, the aliquots were inoculated with the novel strain DSM9906 (KCCM-10168) by use of a sterilized metal loop. They were incubated at 30℃ for 20 hours with shaking at 120 cycles per min.

• Production Culturing : 70 mℓ aliquots of the threonine production media were placed in 500 mℓ Sakaguchi flasks and autoclaved at 121℃ for 15 min. under pressure. After being cooled, the aliquots of the autoclaved threonine production media were inoculated with the pre-cultures of DSM9906(KCCM-10168) at a level of 1%. The strain was incubated at 30℃ for 72 hours with shaking at 120 cycles per min. After fermentation, L-threonine was found to be accumulated at an amount of 22.23 mg/mℓ in the novel strain DSM9906(KCCM-10168) culture. When the above procedure was performed using the parent strain KCCM-10132, L-threonine was found to be accumulated at an amount of 13.09 mg/mℓ.

Example 2

• Strain used : DSM9906 (KCCM-10168)

• Primary pre-culture medium composition : Same as the Pre-culture medium composition of Example 1.

• Secondary Pre-culture medium composition : Glucose 2%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ℓ, Manganese sulfate 2 mg/ℓ, Ammonium sulfate 0.05%, Urea 0.6%, L-Methionine 200 mg/ℓ, pH 7.0

• Production medium composition : Glucose 10%, Corn steep liquor 3%, Potassium dihydrogen phosphate 0.1%, Ferrous sulfate 2 mg/ℓ, Manganese sulfate 2 mg/ℓ, Ammonium sulfate 0.05%, Urea 0.6%, L-Methionine 200 mg/ℓ and pH 7.0. In the case of

the parent strain KCCM-10132, L-Isoleucine was added at 200 ㎎/ℓ.

• Pre-Culturing : A primary pre-culture of DSM9906(KCCM-10168) was obtained in the same manner as that of Example 1. It was inoculated at 1% in 50 ㎖ aliquots of the secondary pre-culture media in Sakaguchi flasks, which had been autoclaved at 121 ℃ for 15min. Incubation was carried out at 30 ℃ for 24 hours with shaking at 120 cycles per min, to give a secondary pre-culture.

• Production Culturing : 2 ℓ of the production media were bottled in a 5 ℓ fermentation bath and then autoclaved at 121 ℃ for 15 min. under pressure. The secondary culture of DSM9906 (KCCM-10168) was inoculated at 5~10% and incubated at 30 ℃ for 100 hours with aeration at 0.8~1.5vvm and stirring at 550 rpm. Glucose were added so as to maintain the glucose concentration of the media at 1~3%. The media were adjusted into pH 6.5~7.0 with ammonia water. After fermentation, L-threonine was found to be accumulated at an amount of 110.20 ㎎/㎖ in the DSM9906 (KCCM-10168) culture. In the same manner as the above, L-threonine was produced from the parent strain KCCM-10132 and measured to be 95.24 ㎎/㎖. 1 ℓ of each of the cultures was centrifuged to harvest the bacteria. The supernatant was passed through an ion exchange resin to adsorb L-threonine, eluted and purified to yield L-threonine crystals at an amount of 104.7 ㎎/㎖ from the culture of DSM9906 and 90.5 ㎎/㎖ from the culture of KCCM-10132. L-Isoleucine 200 ㎎/㎖ was added in the secondary pre-culture medium and production culturing of the parent strain KCCM-10132.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

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RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : Escherichia coli DSM9906	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM 10168
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: [ ] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on July. 22. 1999 (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authouized official(s): Date: July. 29. 1999

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authouity.

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WHAT IS CLAIMED IS:

1. An L-threonine-producing microorganism having characteristics comprising: requirement of L-methionine for growth; L-isoleucine-leaky for growth; resistance to α-methylserine, diaminosuccinic acid, L-glutamic acid, L-threonine, fermentation mother liquid containing L-threonine, azetidine and dehydroproline; and susceptibility to fluoropyruvate.

2. The microorganism of Claim 1, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 50 mg/ℓ to about 400 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 10mM to about 200mM; growth in medium comprising diaminosuccinic acid at a concentration from about 0.5g/L to about 50g/L; growth in medium comprising L-glutamic acid at a concentration from about 50mM to about 500mM; growth in medium comprising L-threonine at a concentration from about 1% to about 13%; growth in medium comprising fermentation mother liquid at a concentration from about 20% to about 80%; growth in medium comprising azetidine at a concentration from about 0.5 g/ℓ to about 5 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 0.5 g/ℓ to about 5 g/ℓ, and inability to grow in medium comprising fluorpyruvate a concentration from about 10mM to about 200mM.

3. The microorganism of Claim 2, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration from about 100 mg/ℓ to about 300 mg/ℓ, than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration from about 20mM to about 100mM; growth in medium comprising diaminosuccinic acid at a concentration from about 1g/L to about 10g/L; growth in medium comprising L-glutamic acid at a concentration from about 100mM to about 300mM; growth in medium comprising L-threonine at a concentration from about 3% to about 10%; growth in

medium comprising fermentation mother liquid at a concentration from about 40% to about 70%; growth in medium comprising azetidine at a concentration from about 1 g/ℓ to about 3 g/ℓ; growth in medium comprising dehydroproline at a concentration from about 1 g/ℓ to about 3 g/ℓ, and inability to grow in medium comprising fluorpyruvate a concentration from about 20mM to about 100mM.

4. The microorganism of Claim 3, wherein said microorganism has characteristics comprising: growth in minimal medium comprising L-methionine at a concentration of about 200 mg/ℓ but not in minimal medium lacking methionine; more robust growth in minimal medium comprising L-isoleucine at a concentration of about 200 mg/ℓ than in minimal medium lacking L-isoleucine; growth in medium comprising α-methylserine at a concentration of about 40mM; growth in medium comprising diaminosuccinic acid at a concentration of about 2.5g/L; growth in medium comprising L-glutamic acid at a concentration of about 240mM; growth in medium comprising L-threonine at a concentration from about 7%; growth in medium comprising fermentation mother liquid at a concentration of about 60%; growth in medium comprising azetidine at a concentration of about 2 g/ℓ; growth in medium comprising dehydroproline at a concentration of about 2 g/ℓ, and inability to grow in medium comprising fluorpyruvate a concentration of about 40mM.

5. The microorganism of any one of Claims 1-4, wherein said microorganism is a strain of *Escherichia coli*.

6. The microorganism of Claim 5, wherein said microorganism has the Korean Culture Center of Microorganisms deposit number KCCM-10168.

7. The microorganism of Claim 5, wherein said microorganism has all the characteristics of the microorganism having the Korean Culture Center of Microorganisms deposit number KCCM-10168.

8. A method of making L-threonine comprising growing a microorganism according to any one of the foregoing claims under conditions in which said microorganism produces L-threonine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00488

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C 12 N 1/20; C 12 P 13/08 // (C 12 N 1/20; C 12 R 1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C 12 N 1/20; C 12 P 13/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZA 9810327 A (DAESANG CORP) 28 July 1999 (28.07.99) abstract Database WPI on EPOQUE, week 199948, London: Derwent Publications Ltd., AN: 199-572381	1-8
A	JP 1039996 A (TORAY IND INC) 10 February 1989 (10.02.89) abstract Database WPI on EPPQUE, week 198912, London: Derwent Publications, Ltd., AN: 1.8.89, 9711.	1-5,8
A	EP 0213536 B1 (TORAY INDUSTRIES, INC.) 18 March 1992 (18.03.92) claims 1,6,7.	1-5,8
A	US 5264353A (YAMADA et al.) 23 November 1993 (23.11.93) column 1, lines 54-68; column 3, line 36 - column 4, line 66; claim 2.	1-5,8

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2000 (14.04.2000)	11 August 2000 (11.08.2000)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer Mosser Telephone No. 1/53424/437

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00488

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
ZA	A	9810327	20-05-1999	WO	A1	00009660	24-02-2000
JP	A2	1039996	10-02-1989			none	
EP	A2	213536	11-03-1987	DE	C0	3684383	23-04-1992
EP	A3	213536	23-03-1988	JP	A2	62198396	02-09-1987
EP	B1	213536	18-03-1992	JP	B4	4068916	04-11-1992
				JP	A2	62044193	26-02-1987
				JP	B4	3013875	25-02-1991
				US	A	5264353	23-11-1993
US	A	5264353	23-11-1993	JP	A2	61201856	06-09-1986
US	A	5264353	23-11-1993				
US	A	5264353	23-11-1993				